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Review

Cholinesterases and the fine line between poison and remedy

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ABSTRACT

Acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8) are related enzymes found across the animal kingdom. The critical role of acetylcholinesterase in neurotransmission has been known for almost a century, but a physiological role for butyrylcholinesterase is just now emerging. The cholinesterases have been deliberately targeted for both therapy and toxicity, with cholinesterase inhibitors being used in the clinic for a variety of disorders and conversely for their toxic potential as pesticides and chemical weapons. Non-catalytic functions of the cholinesterases (ChEs) participate in both neurodevelopment and disease. Manipulating either the catalytic activities or the structure of these enzymes can potentially shift the balance between beneficial and adverse effect in a wide number of physiological processes.

1. Introduction

An often paraphrased statement by the 16th century Swiss physician and philosopher Paracelsus is that "dose separates poison from remedy". Students in basic pharmacology and toxicology learn early on of the 'therapeutic index", a quantitative relationship between efficacy and toxicity and a direct conceptual descendent of Paracelsus' edict. The importance of dose-response relationships in pharmacology and toxicology is difficult to overstate, as they provide chemical-specific views of drug and toxicant potency, efficacy, and selectivity. The ChEs, acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.18) are related enzymes expressed throughout much of the animal kingdom. AChE plays a well-defined role in regulating cholinergic signaling while the physiological impact of BChE has remained unclear until very recently. Here we provide a brief overview of the biology of ChEs and consider how drug- or toxicant-induced changes in their esterase activity, or in the proteins themselves, can shift the balance between benefit and harm.

2. Two cholinesterases

Acetylcholinesterase (AChE) and its "sister" enzyme butyrylcholinesterase (BChE) are widely expressed throughout the animal kingdom [1]. AChE and BChE share roughly 50% sequence homology and have relatively similar tertiary and quaternary structures. They both possess a catalytic triad of three amino acids (serine, glutamate and histidine) located deep inside a "gorge" in the tertiary structure [2–4]. Evidence suggests that these enzymes emerged from a carboxylesterase superfamily, with "true" AChE first emerging hundreds of millions of years ago in Platyhelminthes [5]. Higher vertebrates have one ACHE gene and one BCHE gene, while some lower species express multiple genes of one or both [1]. The cyclostomes, jawless fish including the lamprey and hagfish, only express AChE, suggesting that BChE arose later in evolution by gene duplication and divergence from AChE [1,2,6].

3. Acetylcholinesterase and cholinergic signaling

The concept of a synapse between a neuron and an innervated cell, and the receptors that mediated their interaction, was developed by Bernard, Ehrlich, Sherrington, Langley and others (see [7–9]. It was long debated whether transmission of nerve impulses to muscle cells occurs by electrical or by chemical signals until the work of Otto Loewi and Henry Dale, later recognized by their Nobel Prize in 1936 [10]. The gains in understanding of various physiological processes by these early investigators and others were aided by using natural toxins. In fact, Loewi [11] considered the primary objective of pharmacology as "revealing physiological functions by the reactions of living matter to chemical agents". While this narrow description does not encompass the multifold aspects of modern pharmacology, the experimental use of xenobiotics has played an essential role in gaining an understanding of neurotransmission and cholinergic signaling.

Over a century ago, Dale [12] compared the effects of selected choline esters with the mushroom toxin, muscarine, and was the first to describe "muscarine-like" and "nicotine-like" actions. The relative potency of choline esters in isolated organ systems *vs* intact animals led

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him to posit that the "evanescence" of their effects could be due to rapid hydrolysis by an esterase. In Loewi's classic studies [13], stimulating the vagus in a nerve-heart preparation in physiological solution triggered release of a substance called *vagusstoff* (i.e., vagus substance) that mimicked the effect of nerve stimulation when the fluid medium was transferred to a second heart with no vagal connection. Importantly, Loewi also showed that the effect of the vagal substance was (like that of acetylcholine) enhanced by eserine, a known inhibitor of ChEs [14,15]. Soon thereafter, Dale and Dudley [16] reported the isolation of acetylcholine from tissue (horse spleen), confirming its endogenous presence. These studies and others laid groundwork for an enormous amount of research on the role of acetylcholine in synaptic signaling and its regulation by AChE.

There is now a widespread consensus that AChE is the paramount or sole enzyme regulating neurotransmission in vertebrate cholinergic pathways that include brain, skeletal muscle and the autonomic nervous system. AChE serves this role in all mammals by selectively inactivating acetylcholine, within seconds or milliseconds after it is released from a presynaptic cholinergic neuron. AChE is one of the most efficient enzymes in the body, with a catalytic rate that approaches the limit of diffusion [17,18]. AChE's function appears equally important in brain and the periphery. This view is supported by the intensely concentrated localization of this enzyme at cholinergic synapses throughout the body, and by the diversity of effects elicited by inhibiting AChE either in the brain or in the peripheral compartment.

4. Physiological role of BChE

In contrast to the long-established and well-defined role of AChE in regulating cholinergic signaling, a true physiological function for BChE remained elusive over many decades. BChE exhibits much broader substrate specificity than AChE. For example it hydrolyzes butyrylcholine and acetylcholine while AChE only hydrolyzes the latter. Also, while BChE expression in many tissues exceeds that of AChE, it exists at much lower concentrations in the brain, skeletal muscle, and peripheral nerves [19]. Although exogenous butyrylcholine has been shown to modulate intrinsic cardiac neuron activity in canines [20,21], to our knowledge no synapses in higher vertebrates use butyrylcholine as a neurotransmitter. In fact, a longstanding consensus holds that such synapses do not exist. Evidence to support that view is that, in our unpublished studies, selective inhibitors such as iso-OMPA (tetra isopropyl pyrophosphoramide) can completely inhibit BChE catalysis without eliciting obvious physiological disturbance. Not surprisingly, BChE knockout mice with no BChE expression appear perfectly healthy [22]. In particular, they show no apparent change in motor, autonomic or cognitive function. Under casual observation they are indistinguishable from wild-type mice. Moreover, there are isolated human populations who have been identified as completely lacking a functional BCHE gene, but again, by all accounts, they exhibit a normal phenotype. Their only physiologic difference from "wild-type" is an elevated risk when exposed to bioactive esters in food or ester-type muscle relaxants in the clinic [23,24].

Thus, until quite recently, BChE was considered to lack an important function apart from serving as a "backup" for AChE in regard to neurotransmission, and as a modestly protective bioscavenger of bioactive esters in the food supply. The latter could be regarded as a feature that enables humans and other species to obtain nutrients from plants, many of which could be toxic if their endogenous esters were not efficiently hydrolyzed. The enzyme's ability to hydrolyze esters has been harnessed in surgical procedures using ester-based pharmaceuticals such as the muscle relaxant succinylcholine, which it rapidly inactivates. However, it became apparent early on that certain patients required exceptionally long recovery times after treatment with succinylcholine. The basis for this difference in clinical response is pharmacogenomic in nature, i.e., these individuals were found to express an "atypical BChE" with active site mutations leading to catalytic efficiency far below that of the native enzyme. Because of this BChE variant, they cleared succinylcholine slowly and needed assisted respiration and clinical surveillance for extended periods.

More recently, it was discovered that BChE hydrolyzes the neuropeptide gut hormone, ghrelin [25–28]. Nonetheless, because the enzyme reaction is very slow, those who first reported this finding were initially reluctant to attribute a real physiological role for that phenomenon. Our own views changed when we accidentally linked high level gene transfer of BChE in group-housed male mice to reduced stress, reduced aggression and reduced levels of plasma ghrelin [29]. We are now confident that ghrelin modulation represents an important physiological role for this enzyme and, in light of that role, there is real potential for using BChE to modulate ghrelin's impact in many types of emotional disorders.

Ghrelin is a 28-amino acid peptide with a serine residue acylated by octanoic acid. This feature is essential for binding and activating its primary target, the G-protein-coupled growth hormone secretagogue receptor (GSHR) [30,31]. The major source of plasma ghrelin derives from endocrine cells in the stomach, which release it into the general circulation. Circulating ghrelin then feeds back on the stomach to stimulate gastric muscles that produce "hunger pangs." It triggers afferent vagal neurons in the stomach to activate CNS regions involved in food seeking. At the same time plasma ghrelin also penetrates the blood brain barrier to stimulate GHSR and drive food cravings. In addition, brain neurons in or near the pituitary gland and hypothalamus produce and release ghrelin locally. Activating GHSR in the pituitary leads to growth hormone secretion, while activation elsewhere plays a role in many other processes including glucose homeostasis and fat storage [32,33]. Because BChE is present in both the bloodstream and the brain, its hydrolytic activity plays a role in regulating ghrelin signaling by cleaving its acyl group to form desacyl-ghrelin, which is the dominant form of the peptide in plasma and CSF [34]. Therefore, changes in blood BChE activity, e.g., in response to an anticholinesterase (anti-ChE), can shift the balance of GHSR signaling in favor of active acyl-ghrelin. However, desacyl-ghrelin also has multiple effects in a GHSR-independent manner. These relationships further highlight the physiological implications of altering ghrelin metabolism by reducing BChE activity with enzyme inhibitors or raising it with BChE gene transfer [29,35–37].

Ghrelin serves as a stimulant for hedonic feeding, promoting food intake and fat storage [38–40]. Healthy lean individuals experience a drive for food in response to ghrelin pulses [38]. Circulating ghrelin levels are influenced by food intake, being high before a meal and low afterwards [41]. The most obvious role for BChE's modulation of ghrelin is to regulate feeding behavior/food intake. Under ordinary conditions, circulating BChE is stable, with little change from hour to hour, day to day, or week to week. In contrast, levels of ghrelin released by the stomach or within the brain can change sharply across time. If BChE levels are too low, the drive to eat could be heightened. It has been reported that obese humans and dogs have modestly higher plasma BChE and lower plasma ghrelin than their lean counterparts [42,43]. Similar findings have been reported in mouse models of obesity [44]. By the same token, when obese humans and mice succeed in recovering their original healthy weight, plasma BChE falls and plasma ghrelin rises, often markedly. Evidence to date thus suggests that the levels of BChE activity and ghrelin activity are inversely coupled. This implies a potential for changes in plasma or tissue BChE to influence ghrelin metabolism and thereby impact the respective signaling pathways of acyl- and desacyl-ghrelin [45]. Following up these insights it seems feasible to manipulate BChE levels to impact ghrelin-driven overeating and obesity [46].

5. Cholinesterases and pharmacological effects of inhibitors in the periphery

It is now known that cholinergic signaling plays a vital role in